METHOD TO PRODUCE AN ANTITUMORAL IMMUNE RESPONSE IN CANCER PATIENTS THROUGH A DOUBLE VACCINATION PREVIOUS TREATMENT OF PATIENTS IN ORDER TO ENHANCE THE TUMOR SPECIFIC ANTIGENICITY

TUMOR ASSOCIATED ANTIGENS (TAAs) STORAGE IN TUMOR CELLS TAAs GENERATION AND PRESERVATION Insulin + DNA targeted chemotherapy (see details in Fig 2)

Protein Synthesis, Mutagenic and Epigenetic Proteins modification, Chaperones Synthesis ENHANCEMENT OF ANTITUMORAL IMMUNE RESPONSE - FIRST STEP-Granulocyte-Macrophage Colony Stimulating Factors (see details in Fig3) Activation of Antigen Presenting Cells or APC ENHANCEMENT OF ANTITUMORAL IMMUNE RESPONSE - SECOND STEP-Cyclophosphamide Before Antigen Exposure (see details in Fig 4) Inhibition of Immune-Tolerance for Tumor Associated Antigens (TAAs) INTERNAL VACCINATION BY RELEASE OF TAAS FROM TUMOR CELLS Ascorbic Acid High Dose (see details in Fig 5) Inducing in Tumor Cells Autoschizis or Immunogenic Apoptosis EXTERNAL VACCINATION BY HEMODERIVATIVE , Arterial blood sample, Sedimentation, Hypotonic/Freezing cytolysis, Thermal fractionation, Membrane Filtration. (see details in Fig 6) **Open TAAs-Chaperone Complexes** with immunogenicity preservation

TUMOR ASSOCIATED ANTIGENS (TAAs) STORAGE IN TUMOR CELLS TAAS GENERATION AND PRESERVATION

Protein Synthesis, Mutagenic and Epigenetic Proteins modification, Chaperones Synthesis. Selectivity of Tumor Cells by High Expression of Receptors for Insulin-like Growth Factors

Days 1-4

Insulin 0.3 U/K Body Weigh

Cyclophosphamide 200 mg, Methotrexate 12.5 mg, Fluorouracil 250 mg

ENHANCEMENT OF ANTITUMORAL IMMUNE RESPONSE - FIRST STEP-Granulocyte-Macrophage Colony Stimulating Factors (see details in Fig3) Activation of Antigen Presenting Cells or APC ENHANCEMENT OF ANTITUMORAL IMMUNE RESPONSE - SECOND STEP-Cyclophosphamide Before Antigen Exposure (see details in 1 ig 4) Inhibition of Immune-Tolerance for Tumor Associated Antigens (TAAs) INTERNAL VACCINATION BY RELEASE OF TAAS FROM TUMOR CELLS Ascorbic Acid High Dose (see details in Fig 5) Inducing in Tumor Cells Autoschizis or Immunogenic Apoptosis EXTERNAL VACCINATION BY HEMODERIVATIVE Arterial blood sample, Sedimentation, Hypotonic/Freezing cytolysis, Thermal fractionation, Membrane Filtration. (see details in Fig 6) Open TAAs-Chaperone Complexes with immunogenicity preservation

TUMOR ASSOCIATED ANTIGENS (TAAs) STORAGE IN TUMOR CELLS TAAS GENERATION AND PRESERVATION

Insulin + DNA targeted chemotherapy (see details in Fig 2)
Protein Synthesis, Mutagenic and Epigenetic Proteins modification, Chaperones Synthesis

ENHANCEMENT OF ANTITUMORAL IMMUNE RESPONSE -FIRST STEP-

Activation of Antigen Presenting Cells or APC
Days 8-12

Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) 150 µg/m²/day, s.c.

ENHANCEMENT OF ANTITUMORAL IMMUNE RESPONSE. -SECOND STEP-Cyclophosphamide Before Antigen Exposure (see details in Fig 4) Inhibition of Immune-Tolerance for Tumor Associated Antigens (TAAs)

INTERNAL VACCINATION BY RELEASE OF TAAS FROM TUMOR CELLS
Ascorbic Acid High Dose (see details in Fig 5)
Inducing in Tumor Cells Autoschizis or Immunogenic Apoptosis

EXTERNAL VACCINATION BY HEMODERIVATIVE
Arterial blood sample, Sedimentation, Hypotonic/Freezing
cytolysis, Thermal fractionation, Membrane Filtration. (see
details in Fig 6)

Open TAAs-Chaperone Complexes with immunogenicity preservation

TUMOR ASSOCIATED ANTIGENS (TAAs) STORAGE IN TUMOR CELLS TAAS GENERATION AND PRESERVATION

Insulin + DNA targeted chemotherapy (see details in Fig 2)
Protein Synthesis, Mutagenic and Epigenetic Proteins modification, Chaperones Synthesis

ENHANCEMENT OF ANTITUMORAL IMMUNE RESPONSE - FIRST STEP-Granulocyte-Macrophage Colony Stimulating Factor (see details in Fig3)

Activation of Antigen Presenting Cells or APC

ENHANCEMENT OF ANTITUMORAL IMMUNE RESPONSE - SECOND STEP-

Inhibition of Immune-Tolerance for Tumor Associated Antigens (TAAs)

By Cyclophosphamide Before Antigen Exposure

Day 5:

Cyclophosphamide 300 mg/m²

INTERNAL VACCINATION BY RELEASE OF TAAS FROM TUMOR CELLS
Ascorbic Acid High Dose (see details in Fig 5)
Inducing in Tumor Cells Autoschizis or Immunogenic Apoptosis

EXTERNAL VACCINATION BY HEMODERIVATIVE
Arterial blood sample, Sedimentation, Hypotonic/Freezing
cytolysis, Thermal fractionation, Membrane Filtration. (see
details in Fig 6)

Open TAAs-Chaperone Complexes with immunogenicity preservation

TUMOR ASSOCIATED ANTIGENS (TAAs) STORAGE IN TUMOR CELLS TAAS GENERATION AND PRESERVATION Insulin + DNA targeted chemotherapy (see details in Fig 2) Protein Synthesis, Mutagenic and Epigenetic Proteins modification, Chaperones Synthesis

> ENHANCEMENT OF ANTITUMORAL IMMUNE RESPONSE - FIRST STEP-Granulocyte-Macrophage Colony Stimulating Factor (see details in Fig3) Activation of Antigen Presenting Cells or APC

> ENHANCEMENT OF ANTITUMORAL IMMUNE RESPONSE -SECOND STEP-Cyclophosphamide Before Antigen Exposure (see details in 1'ig 4) Inhibition of Immune-Tolerance for Tumor Associated Antigens (TAAs)

INTERNAL VACCINATION BY RELEASE OF TAAS FROM **TUMOR CELLS**

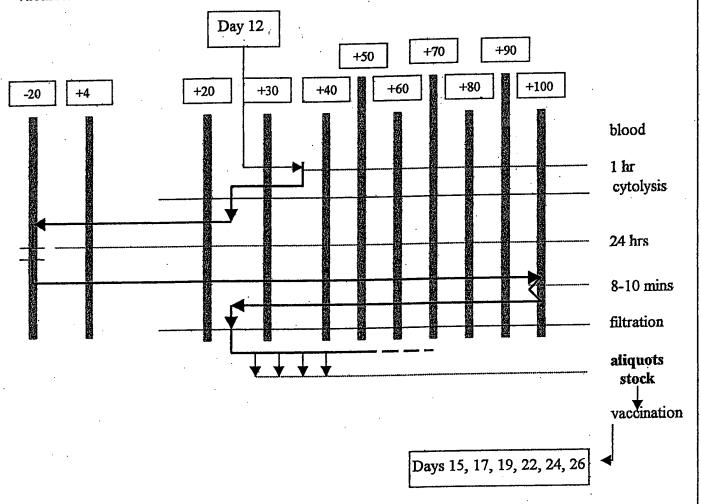
Inducing in Tumor Cells Autoschizis or Immunogenic Apoptosis Days 8-12

Ascorbic Acid 25 gm /250 ml 1/2 Lactate-Ringer/i.v. 50 min Variation + Menadione 250 mg, i.v.

EXTERNAL VACCINATION BY HEMODERIVATIVE Arterial blood sample, Sedimentation, Hypotonic/Freezing cytolysis, Thermal fractionation, Membrane Filtration. (see details in 1'ig 6) Open TAAs-Chaperone Complexes with immunogenicity preservation

EXTERNAL VACCINATION BY HEMODERIVATIVE

Arterial blood sample, Sedimentation, Hypotonic/Freezing cytolysis, Thermal fractionation, Membrane Filtration in order to obtain a composition for sub-cutaneous vaccination with TAAs released from SSP complexes with immunogenicity preserved.



ALL TEMPERATURES ARE EXPRESSED IN $\,^{0}\mathrm{C}\,$ (CELSIUS DEGREES)